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REGIONAL DISTRIBUTION OF MUSCLE FIBER TYPES IN THE ASYMMETRIC CLAWS OF CALIFORNIAN SNAPPING SHRIMP

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Abstract

The properties of the opener and closer muscles in the asymmetric claws of *Alpheus californiensis* have been investigated using sarcomere length measurements and histochemical techniques. In the smaller pincer claw two types of muscle fibers are regionally distributed within the single closer muscle. A central band of fibers have short (2.5 μm) sarcomeres and high myofibrillar ATPase activity. Intermediate-type fibers have smaller diameters, sarcomeres 8.5 to 9 μm in length and low myofibrillar ATPase activity. The snapper closer muscle, by contrast, is composed of fibers with long (11–14 μm) sarcomeres and low myofibrillar ATPase activity. Opener muscle fibers in the pincer claw have shorter sarcomere lengths than their counterparts in the snapper claw.

Introduction

In certain crustaceans, claw dimorphism is accompanied by an asymmetry of claw muscle properties. For example, in lobsters (*Homarus americanus*) the rapidly closing cutter claw has a large proportion of fast closer muscle fibers, while the slowly closing crusher claw is composed of a uniform population of slow muscle fibers (Govind and Lang, 1974; Lang *et al.*, 1977). In addition it has been shown recently that a similar asymmetry of fiber properties is present between the claw opener muscles (Govind *et al.*, 1981).

In the dimorphic claws of snapping shrimp (*Alpheus*) differences exist in claw closer muscle properties (Stephens and Mellon, 1979). In *A. heterochelis* and *A. armillatus* there are three muscles in each claw: a single opener, a minor closer, and a main closer muscle (Ritzmann, 1974). Analysis of sarcomere lengths, used as an indication of muscle fiber contraction properties (Atwood, 1973, 1976; Josephson, 1975), reveals that differences occur only in the main closer muscle. In the larger snapper claw the main closer muscle is composed of a uniform population of slow fibers with long (10–15 μm) sarcomeres. In the smaller pincer claw the main closer muscle has two populations of fiber types. Fast fibers in the central portion of the main closer muscle have relatively large diameters and short (2 and 3 μm) sarcomeres. Intermediate-type muscle fibers have sarcomeres that measure between 6 and 8 μm and are located on the medial and lateral margins of the muscle.

A fascinating feature of adult snapping shrimp is an ability to reverse claw configuration (Wilson, 1903; Przibram, 1931; Mellon and Stephens, 1978). Removal or denervation of the snapper claw causes the remaining pincer to become transformed into a new snapper claw, while a pincer regenerates at the site of the old snapper claw. Pincer–snapper transformation involves a change in the properties of the main closer muscle fibers from fast and intermediate in the pincer to slow in the snapper (Stephens and Mellon, 1979).

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Measurement of sarcomere length is one method used to examine the properties of single fibers in a particular muscle. A major disadvantage with this technique is the difficulty in constructing a complete picture of the properties of all of the fibers in a given muscle. A new technique, however, allows differentiation of fast and slow crustacean muscle fibers based on histochemistry (Ogonowski and Lang, 1979; Silverman and Charlton, 1980). In a previous paper we showed that in *A. californiensis* the sarcomere lengths of the single claw closer muscle are similar to those described for the main closer muscle of *A. heterochelis* (Stephens *et al.*, unpublished observations). In the present study we have used histochemistry and sarcomere length measurements to investigate the properties of the closer and opener muscles in dimorphic claws of *A. californiensis*.

Materials and Methods

Snapping shrimp (*Alpheus californiensis*) were obtained commercially from Venice, California, and were retained individually in constantly circulating, artificial sea water at 14°C. The animals were fed Tetramin twice weekly, and under these conditions lived for at least 3 months in the laboratory.

Sarcomere length measurements

Sarcomere length measurements were made from opener or closer muscles in pairs of claws removed from adult shrimp. One of the claw muscles was carefully dissected away and the remaining muscle was fixed at resting length; the closer muscle was fixed with the dactyl in the open position, and the opener muscle with the dactyl in the closed position (Lang *et al.*, 1977; Stephens and Mellon, 1979). To prevent measurement errors due to muscle contraction (Meiss and Govind, 1979), the dissected claws were soaked for 60 minutes in a snapping shrimp saline in which calcium ions had been replaced with magnesium. The claws were then fixed for 2 days in alcoholic Bouin's solution. Individual muscle fibers were carefully teased apart in 80% ethanol on a microscope slide, and examined using a compound microscope fitted with Normarski optics. The length of five successive sarcomeres was measured using a calibrated filar ocular micrometer. At least three measurements were made for each muscle fiber and the average length of a single sarcomere was calculated.

Histochemistry

Certain histochemical properties of the muscle fibers in the dimorphic claws were examined. Fully developed pincer and snapper claws were removed from adult shrimp, quickly frozen in liquid nitrogen, and sectioned on a cryostat microtome. Transverse sections (15 μm thick) from pairs of claws were mounted on glass cover slips and the myofibrillar adenosinetriphosphatase (ATPase) activity was determined using a technique used for lobsters (Ogonowski and Lang, 1979)—a modification of conventional methods for vertebrate tissue (Padykula and Hermann, 1955). A more recently published technique for determining myofibrillar ATPase activity (Silverman and Charlton, 1980) was employed with less success.

Results

Sarcomere length measurements

Sarcomere length measurements were made from closer and opener muscle fibers in pairs of fully developed claws removed from the same animal. The closer

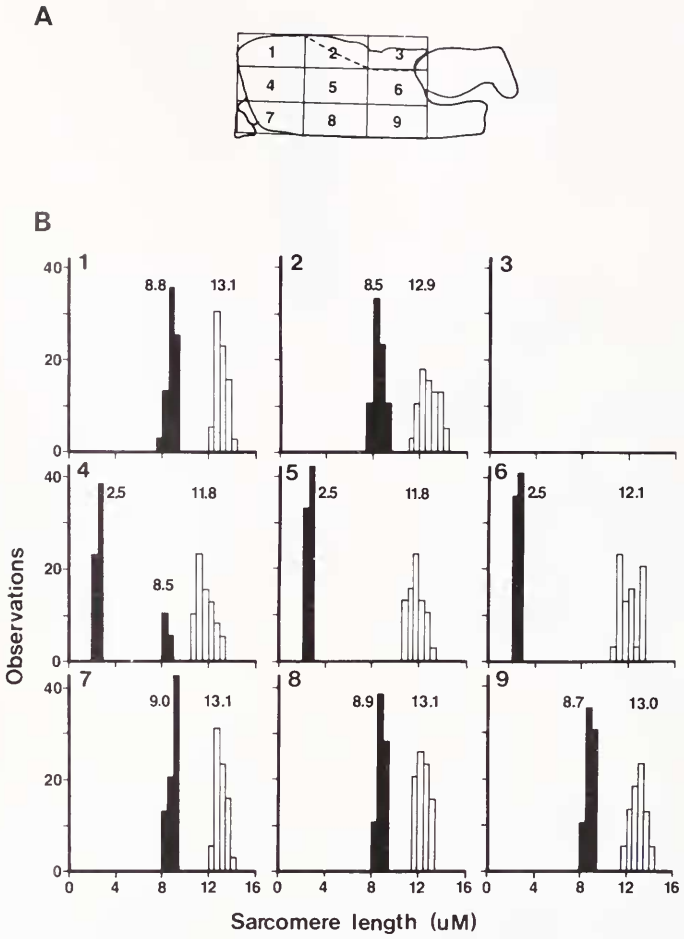

FIGURE 1. The regional distribution of sarcomere lengths in the claw closer muscle. (A) Diagram of a snapper claw with the propus divided into 9 regions. (B) Histograms of sarcomere length data for closer muscle fibers removed from each region (1–9) in a pincer (filled columns) and snapper (open columns) claw removed from the same animal. Inset numbers represent mean sarcomere length values.

muscle was divided by eye into 9 regions (Fig. 1A) and muscle fibers were carefully removed from the central portion of each region. It should be noted that observations were not made from fibers in region 3 since only opener muscle is present in that region. In the smaller pincer claw, closer muscle fibers located in the dorsal (1 and 2) and ventral (7 to 9) regions are made up of sarcomeres with mean lengths of 8.5 to 9.0 μm (Fig. 1B). The central regions (4 to 6), by contrast, contain muscle fibers with mostly short (2.5 μm) sarcomeres. A similar regional distribution of different fiber types has been reported for the pincer main closer muscle of *A. armillatus* (Stephens and Mellon, 1979).

In the larger snapper claw, the closer muscle fibers are composed of long sarcomeres (Fig. 1B). Fibers located in the central regions of the muscle have sarcomere lengths that are shorter than those in the dorsal and ventral regions, however these differences are not statistically different (Student's *t*-test).

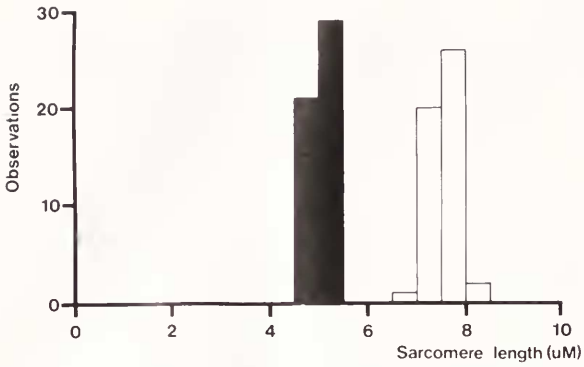

FIGURE 2. Histograms to show the sarcomere lengths of opener muscle fibers in a pair of pincer (filled columns) and snapper (open columns) claws from the same animal.

Opener muscle fibers from pincer and snapper claws are composed of sarcomeres of different lengths. In the example given in Figure 2, opener muscle sarcomeres have mean lengths of 7.5 μm and 5.0 μm, respectively, for the snapper and pincer. No regional differences in sarcomere length were observed in the opener muscle of either claw.

Histochemistry

Figure 3 shows photomicrographs of frozen transverse sections taken from a pair of claws and stained for myofibrillar ATPase activity. Sections of snapper claws showed uniform light staining profiles for opener and closer muscle fibers (Fig. 3C). In sections of the pincer claw, however, a central band of closer muscle fibers was always darkly stained (Figs. 3D–F), indicating a higher ATPase activity in these fibers than in those located in the dorsal and ventral regions. Using this same technique on lobsters, Ogonowski and Lang (1979) showed that muscle fibers with high myofibrillar ATPase activity are rapidly contracting, fast muscle fibers. The location of the dark-staining closer muscle fibers in the pincer claw (Fig. 3) correlates well with the location of short sarcomere fibers (Fig. 1), indicating that there is a central band of fast fibers. In the pincer claw of *A. armillatus* the fast main closer muscle fibers have a larger diameter than the intermediate muscle fibers (Stephens and Mellon, 1979). In the present study myofibrillar ATPase activity was used to differentiate between fast and intermediate fibers in the pincer closer muscle. Figure 4 shows closer muscle fiber diameter data for the light- and dark-staining fibers in transverse sections of a pincer claw, and for closer muscle fibers in the contralateral snapper claw. Although there is no statistical difference between the diameters of the two types of pincer closer muscle fibers, it is apparent that the dark staining fibers in the central region of the claw have a slightly larger diameter than the light-staining fibers in the ventral and dorsal regions. Furthermore, the closer muscle fibers in the snapper are about twice the diameter of their counterparts in the pincer claw.

DISCUSSION

In many crustacean neuromuscular preparations there is a correlation between the speed of muscle contraction, sarcomere length (Atwood, 1973, 1976; Govind

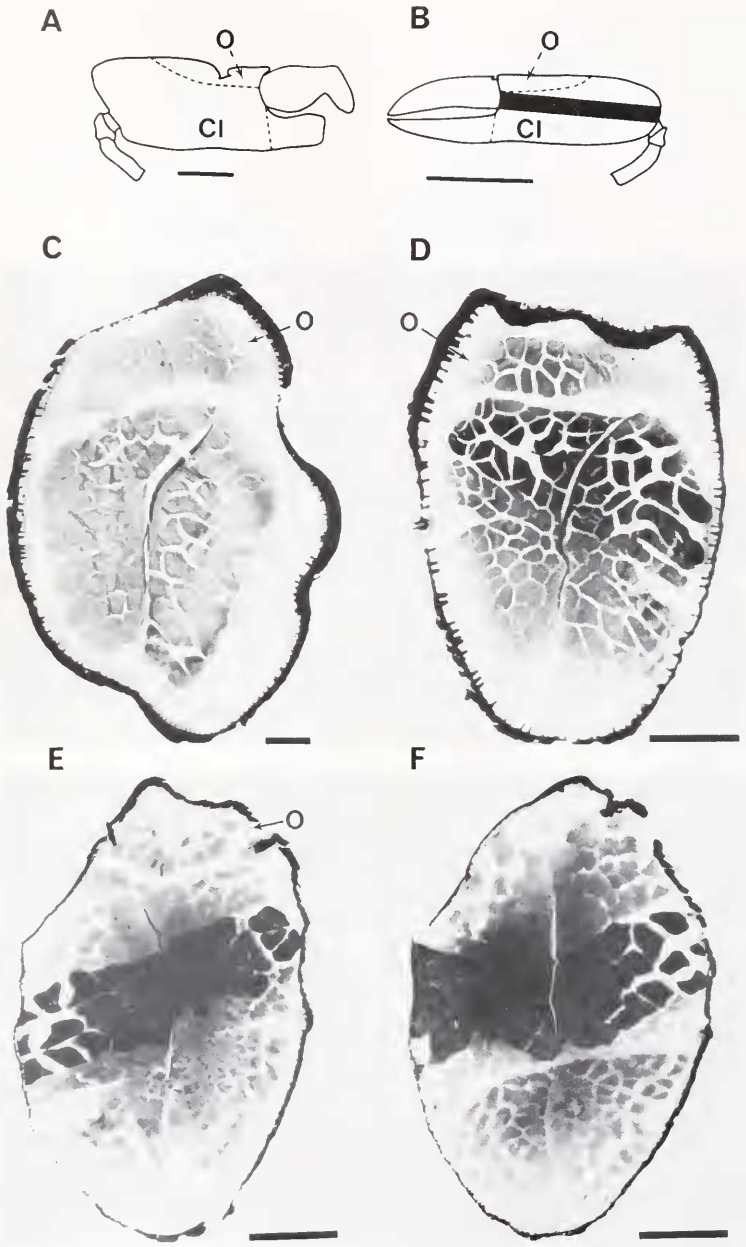

FIGURE 3. Myofibrillar ATPase activity of the claw muscles.

(A,B): Diagrams of a snapper (A) and pincer (B) claw showing the locations of the opener (O) and closer (Cl) muscles. The dark band in the pincer closer muscle represents the location of the fibers with high myofibrillar ATPase activity.

(C–F): Myofibrillar ATPase activity of the claw muscles in frozen transverse sections (15 μm thick) of a snapper (C) and a pincer (D–F) claw. A band of fibers with high myofibrillar ATPase activity is present in the pincer closer muscle. Sections D, E, F were taken distally, centrally, and proximally, respectively, through the propus of the pincer.

Calibration: 1mm (A,B) and 500 μm (C–F).

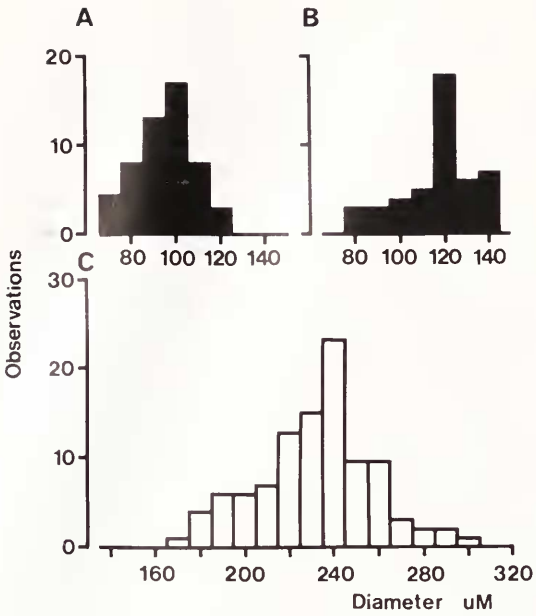

FIGURE 4. The diameter of closer muscle fibers in a pincer (A, B) and snapper (C) claw. Measurements were made from frozen transverse sections of a pair of claws. Data is given for pincer fibers with low ATPase activity (A), high ATPase activity (B), and for snapper closer muscle fibers.

and Lang, 1974; Josephson, 1975) and myofibrillar ATPase activity (Ogonowski and Lang, 1979; Ogonowski *et al.*, 1980; Silverman and Charlton, 1980). Rapidly contracting muscle fibers have short sarcomeres and high myofibrillar ATPase activity, while slow muscle fibers have long sarcomeres and low myofibrillar ATPase activity. Thus in the pincer claw of *A. californiensis*, the fibers in the central region of the closer muscle are presumably fast, while those located on the dorsal and ventral surfaces of the closer muscle are presumably intermediate speed fibers (Figs. 1 and 3). A histological examination of fixed claws from *A. armillatus* revealed similar results for the pincer main closer muscle (Stephens and Mellon, 1979). In addition, it was shown that the centrally located fast muscle fibers have a larger diameter than the intermediate fibers. The possibility that these centrally located muscle fibers contracted immediately prior to fixation, producing a decreased sarcomere length and an increased fiber diameter, could have produced erroneous results (C. Phillips, personal communication). However the present investigation, using frozen sections and also prolonged soaking in calcium-free saline prior to fixation to prevent muscle contraction, produced a similar regional distribution of closer muscle fiber types, without major differences in muscle fiber diameter. Furthermore, we have taken transverse sections of claws of *A. californiensis* following the procedure of Stephens and Mellon (1979) and have observed no clear regional differences in the diameter of pincer closer muscle fibers (unpublished observations). Moreover it is interesting that a distinct band of fast muscle fibers has been found in the central region of the closer muscles of both claws of larval homarid lobsters (Ogonowski *et al.*, 1980). During normal development the closer muscle of the larger crusher claw becomes uniformly slow, while the cutter claw closer muscle retains the dimorphism of fiber types in the adult (Lang *et al.*, 1977).

In the absence of direct measurements, histochemical and histological properties of muscle fibers can provide an indication of contraction speed. In many crustacean muscles, short sarcomere fibers with high myofibrillar ATPase activity are fast, while long sarcomere fibers with low ATPase activity are slow (Atwood, 1973, 1976; Josephson, 1975; Ogonowski and Lang, 1979; Silvermann and Charlton, 1980). From this evidence it appears that the pincer closer muscle is composed of fast and intermediate fibers, while the snapper closer muscle consists of fibers that contract slowly but produce large amounts of tension. This is consistent with behavioral observations made on snapping shrimp (Ritzmann, 1974; Schein, 1975). The pincer claw is used for manipulation of small objects while the snapper claw is used only during territorial encounters with conspecific shrimp. The dactyl initially moves to open the snapper claw and, in Californian snapping shrimp, a pair of discs on the propus and the dactyl become opposed (Ritzmann, 1973). The closer muscle then develops tension to overcome the adhesive force between the discs. The dactyl rapidly closes and causes a jet of water to be projected towards the intruder and also produces the characteristic snapping sound.

The sarcomere length values for the single snapper closer muscle of *A. californiensis* (Fig. 1B) are similar to those reported for the main closer muscle of *A. armillatus* (Stephens and Mellon, 1979). Furthermore, examination of *A. armillatus* with claws undergoing pincer–snapper transformation revealed that the fast and intermediate main closer muscle fibers in the pincer change to slow muscle fibers during this normal developmental process. If, in *A. californiensis*, the differences in the properties of the closer muscle fibers in pairs of claws represent the changes that take place as a pincer transforms into a new snapper, it is apparent that there are similar changes in the closer muscle fiber properties in the two species. However, we have shown recently that the differences in motor axon synaptic facilitation reported for *A. armillatus* (Stephens and Mellon, 1979) are not present in *A. californiensis* (Stephens *et al.*, unpublished observations). Examination of facilitation, using pairs of junctional and synaptic potentials evoked by stimulation of the excitatory axon, showed no facilitation in the snapper closer muscle. In fact, synaptic depression was recorded at short intervals (<100 ms). These data, together with the observation that the closer muscle fibers in either claw appear to be supplied by only one excitor axon, has raised the intriguing possibility that claw transformation may involve some reorganization of peripheral motor axon patterns, as seen in many vertebrate preparations (Rotshenker and McMahon, 1976; Brown and Ironton, 1977; Hubel *et al.*, 1977; Jackson and Diamond, 1979; Rotshenker and Reichert, 1980).

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